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EMBRYOTOXIC AND TERATOGENIC ACTION OF 5-HYDROXYTRYPTAMINE: MECHANISM OF ACTION IN THE RAT

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5-Hydroxytryptamine (5-HT) injected into pregnant mice, rats or rabbits near to term kills the foetuses (Correll, Lyth, Long & Vanderpoel, 1952; Waugh & Pearl, 1960; Poulson, Botros & Robson, 1960a; Ibrahim, 1961; Pepeu & Giarman, 1962). Death usually occurs within 30 min of the injection (Poulson, Botros & Robson, 1960b), but the 5-HT level in the foetus remains unchanged (Robson & Senior, 1964). Intra-amniotic (Ibrahim, 1961; Craig, 1966) or intra-foetal injections of 5-HT (Robson & Sullivan, 1963, 1966) in corresponding doses are not lethal, and this seems to indicate a maternal site of action.

Waugh & Pearl (1960) suggested that 5-HT produced circulatory failure at the utero-placental site in the rat. Erspamer (1961) showed that 5-HT is a powerful vasoconstrictor in the perfused human placenta, and others demonstrated that it constricts the vessels of the umbilical cord in the human (Panigel, 1962) and the rabbit (Pepeu & Giarman, 1962). Robson & Sullivan (1963, 1966) showed that 5-HT produced a marked delay in the passage of radiosodium from the maternal blood into the mouse placenta and foetus. Blood flow in the placenta was shown to be reduced by 5-HT for a period up to 2 hr depending on the dose, and it was suggested that its lethal action was essentially due to interference with chorioallantoic placental function. This is supported by the findings of Craig (1966) in rats.

When 5-HT is administered in a single dose to pregnant mice during the period of embryogenesis (Poulson, Robson & Sullivan, 1963), or daily throughout pregnancy in rats (Reddy, Adams & Baird, 1963) it produces congenital abnormalities in some of the surviving foetuses. Similar abnormalities are induced at the stage of embryogenesis by short periods of anoxia of the mother (Ingalls, Curley & Prindle, 1952) or of the foetus (Brent & Franklin, 1960) in mice and rats respectively. These findings led us to investigate the possibility that 5-HT may produce its lethal and teratogenic effects during the period of embryogenesis by interfering with the supply of nutrients to the developing embryo even though the chorioallantoic placenta has not yet developed at this stage of pregnancy.

METHODS

Female albino Wistar rats weighing about 200–300 g and bred in the Animal House of Guy's Hospital Medical School were used. After overnight pairing with males, mating was confirmed by

the presence of a copulation plug or of sperms in the vagina. This day was called day 1 of pregnancy.

Lethal and teratogenic activity of 5-HT

On the afternoon of day 10 or day 11 of pregnancy the rats were injected subcutaneously with 80, 40, 20, 10, 5 or 2 mg/kg 5-HT creatinine sulphate. The rats were killed with chloroform on day 21 and the uterus was removed and opened. Uteri with no signs of implantation were discarded. Sites of foetal death were recorded as (i) death before day 12 or (ii) death after day 11. In the first case the resorption sites were small white scars in the endometrium. In the second case, where embryonic death had occurred after the formation of the chorioallantoic placenta, the placentae were usually still present in the uterine lumen on day 21 and were of varying sizes depending on the time of foetal death but the foetuses were often completely resorbed. All live foetuses were carefully separated from the amniotic sacs and placentae and examined for gross external and internal abnormalities. Subsequently the skeletons were examined for abnormalities using Dawson's method of alizarin staining (Gatenby & Beams, 1950).

Effect of 5-HT on ^{22}Na equilibration in embryonic and maternal tissues

Experiments were carried out on day 10 or day 11 of pregnancy. Rats were anaesthetized with pentobarbitone 25 mg/kg intraperitoneally for the whole of the experiment. Pregnancy was confirmed by laparotomy and the rats were then injected subcutaneously with 80 or 10 mg/kg 5-HT creatinine sulphate, or with an equivalent volume of normal saline (controls).

3–5 μc ^{22}NaCl (0.5 ml.) were injected into a tail vein, 5, 30 or 90 min after the 5-HT injection. A 0.5 ml. blood sample was taken from the heart 5 or 30 min later and the rat was then killed immediately and bled out (Fig. 1). A number of tissue samples were then removed as quickly as possible.

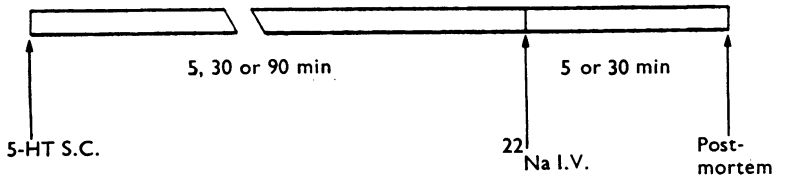

Fig. 1. Scheme of experiments.

First of all both uterine horns were excised and trimmed of all mesometrial blood vessels. Each horn was blotted dry, opened along one side of the anti-mesometrial border and separated into three parts—that is, (1) embryo plus decidua capsularis, (2) decidua basalis and (3) the remaining myometrium and endometrium (Fig. 2). The embryo and decidua capsularis were easily separated from the decidua basalis, but the latter had to be cut away from the uterus. All the embryo and decidua capsularis samples from each horn (approximately 6 per horn) were grouped to make up one sample, as were the decidua basalis samples. The remaining horns (endometrium and myometrium) were also kept separate. Thus two samples each of (1) embryo and decidua capsularis, (2) decidua basalis and (3) endometrium and myometrium were obtained from each rat. One piece each of anterior abdominal muscle, spleen and kidney was removed from each rat just after the excision of the uterus.

Any tissues which were wet or were contaminated with blood were blotted dry. All the tissues were weighed on a 500 mg torsion balance as they were removed, and were then placed at the bottom of test tubes for counting. All the tissues were placed on pre-weighed squares of filter paper (0.75 cm) before weighing to ensure that no material was lost and to facilitate easy and complete transfer to the bottom of the test tube.

Radioactivity was measured using a well crystal scintillation counter and expresed as counts/min (cpm)/g tissue. The radioactivity in the tissues was expressed as a percentage of the radioactivity

in the blood (cpm/g tissue × 100 ÷ cpm/ml. blood). The values for the 5-HT treated groups were compared with those of the saline controls.

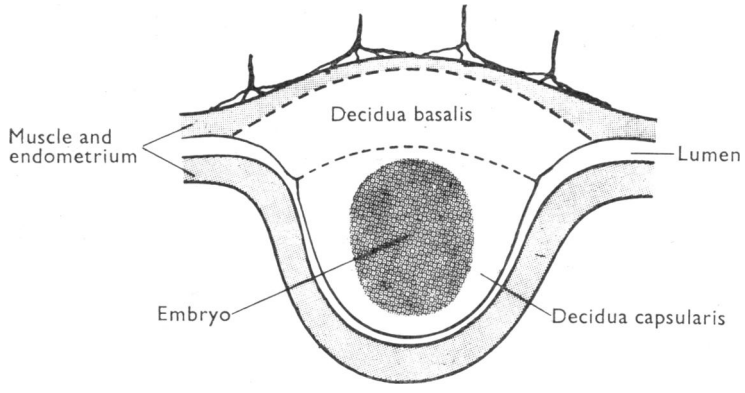

Fig. 2. Diagrammatic representation of a longitudinal section of the rat uterus on day 10 of pregnancy showing an implantation site and the various tissues sampled.

Drugs

^{22}NaCl was obtained from the Radiochemical Centre, Amersham, in normal saline. 5-HT creatinine sulphate (May & Baker Ltd.) was dissolved in distilled water on the day of injection and injected subcutaneously in a dose volume of 4 ml./kg. All doses of 5-HT refer to the weight of the salt, which contains 43.5% of 5-HT base.

RESULTS

In order to study the mechanism of the embryotoxic and teratogenic actions of a single injection of 5-HT, preliminary experiments were carried out to find (1) a dose of 5-HT which would kill all the foetuses on the day of the injection and (2) a dose which would damage rather than kill the foetuses, and thus lead to foetal abnormalities which could be observed at term. This was done in rats on days 10 or 11 of pregnancy, using a wide range of doses of 5-HT (2–80 mg/kg 5-HT creatinine sulphate). Doses of 10 mg/kg and 80 mg/kg of the salt were subsequently tested for their effect on the passage of intravenously injected ^{22}Na into maternal and embryonic tissues. This was used as an index of the nutritional status of the tissues.

Lethal and teratogenic activity of 5-HT

None of the mothers died as a result of any of the 5-HT injections. The effect of 5-HT given on day 10 or 11 of pregnancy is shown in Table 1. There were no live foetuses in 10 pregnant rats given doses of 5-HT greater than 20 mg/kg, and only early resorption sites—that is, pre-day 12—were found in the uterus. In the five rats given 20 mg/kg on day 10 there were no live foetuses, but there was a mean of 2.0 placentae per rat (post day 11 death) indicating that some embryos had survived long enough for the chorio-allantoic placenta to develop, but had subsequently died.

With lower doses of 5-HT many embryos survived until day 21. However the survival rate depended to some extent on whether the 5-HT was given on day 10 or on day 11. Thus with doses of 10 and 5 mg/kg, only 4/10 and 4/6 of the rats respectively had live

foetuses when the treatment was given on day 10, whereas 5/5 and 7/7 rats had live foetuses when treated with 10 and 5 mg/kg 5-HT on day 11. Combining the results for the two dose levels the difference in response for the two days was highly significant ($P<0.005$).

TABLE 1

THE EFFECT OF 5-HT GIVEN AS A SINGLE INJECTION DURING THE PERIOD OF EMBRYOGENESIS (DAY 10 OR 11)

Foetuses were examined on the twenty-first day of pregnancy

Day of injection	Dose (mg/kg)	Fraction of mothers with live foetuses	Pre-day 12 resorption sites (mean)	Post-day 11 resorption sites (mean)	Fraction of foetuses abnormal
	80	0/5	11·2	0	—
	40	0/3	11·0	0	—
10	20	0/5	10·8	2·0	—
	10	4/10	6·5	2·7	13/18
	5	4/6	2·5	0·8	3/27
	2	5/5	0·5	0	3/35
	80	0/2	12·0	0	—
11	10	5/5	7·2	1·0	20/20
	5	7/7	1·4	0·7	22/55
	2	7/7	1·3	0	6/68
10 and 11	Saline	4/4	0·5	0	0/30
	No treatment	10/10	1·0	0·1	1/84

After treatment with 10 mg/kg on day 10 each rat had an average of 2.7 post day 11 resorption sites. A large fraction of the surviving foetuses (13/18) had one or more gross abnormalities such as exencephalus, hydrocephalus, anophthalmia, microphthalmia, shortened snout with protruding tongue, or haemorrhages of the spine or cranium (Table 2).

Treatment with 10 mg/kg on day 11 produced an average of 1.0 post day 11 resorption sites per rat but every surviving foetus (20/20) was abnormal in some way. There were also many abnormal foetuses in the rats treated with 5 mg/kg on day 11 (22/55). The abnormalities in the foetuses from mothers treated on day 11 resulted from the fusion of centra and neural arches in the vertebrae and were apparent only after alizarin staining. The ribs were usually fused, stunted or missing altogether, and in some foetuses the sternebrae were fused in an irregular manner as well. Each foetus usually had many abnormalities in the thoracic region. In addition one foetus had no tail, and another had only two digits on one forepaw.

After a dose of 5 mg/kg on day 10, 3/27 foetuses were abnormal on day 21, whereas 22/55 foetuses were abnormal when the 5-HT was given one day later (Tables 1 and 2). This difference is significant ($P=0.005$). An injection of 2 mg/kg 5-HT on day 10 produced sternal abnormalities in 3/35 foetuses, and when given one day later 6/68 foetuses had abnormalities of the ribs or failure of ossification of parietal bones.

No abnormalities were seen in 30 foetuses from 4 control rats injected with saline on days 10 or 11 or in 84 foetuses from 10 untreated mothers except for one foetus in the untreated group which had a retro-orbital haemorrhage.

TABLE 2

ABNORMALITIES PRODUCED BY A SINGLE INJECTION OF 5-HT ON DAY 10 OR 11 OF PREGNANCY

Dose of 5-HT (mg/kg)	Fraction of foetuses abnormal	Runt	Exencephalus	Hydrocephalus	An- or microphthalmia	Short snout	Retarded ossification of parietals	Spine, rib or sternal deformity	Limb or tail deformity	Hydrops foetalis	Haemorrhage in head or spine
Day 10											
10	13/18	4	2	3	7	3	1	1	—	1	2
5	3/27	—	—	—	1	—	—	2	—	—	—
2	3/35	—	—	—	—	—	—	3	—	—	—
Day 11											
10	20/20	—	—	—	—	—	—	20	1	—	—
5	22/55	7	—	—	—	—	—	17	1	2	—
2	6/68	—	—	—	—	—	5	2	—	—	—
Control Days 10 and 11	1/114	—	—	—	—	—	—	—	—	—	1

Mechanism of the lethal and teratogenic action of 5-HT.

It was shown above that a dose of 80 mg/kg 5-HT killed the embryos on the day of injection into the mother whereas a dose of 10 mg/kg caused much less interference with foetal viability but gave rise to many abnormalities in the surviving foetuses.

We then investigated to what extent, if any, these two dose levels of 5-HT affected the ability of nutrients to pass into various maternal and embryonic tissues. This was done by studying the ability of intravenously injected ^{22}Na to equilibrate with the tissues under normal conditions and in the presence of 5-HT.

Control rats

In the control rats which received only saline subcutaneously, intravenously injected ^{22}Na passed very quickly into the body tissues. Indeed it appears that in the tissues investigated full equilibration occurred within 5 min, because the radioactivity was not significantly different whether the tissues were sampled 5 or 30 min after the ^{22}Na injection.

There were slight but significant differences in ^{22}Na contents of the embryo and decidua capsularis ($P<0.01$), decidua basalis ($P<0.002$) and endometrium and myometrium ($P<0.02$) on day 10 and day 11 respectively (Table 3). No significant differences were found in the ^{22}Na content of abdominal muscle, spleen or kidney samples on these two days, and hence the values have been combined (Table 4). The ^{22}Na contents of the muscle and spleen were smaller than those of other tissues which is accounted for by differences in the extracellular fluid volume.

TABLE 3

THE EFFECT OF 5-HT ON TISSUE ^{22}Na CONTENT (CPM/G×100÷CPM/ML. BLOOD) 5 MIN AFTER AN INTRAVENOUS INJECTION OF $^{22}NaCl$ ON DAY 10 OR 11 OF PREGNANCY

Significance determined by Student's *t* test, each mean being compared with its own control. $P<0.05$*, <0.01†, <0.001‡

Dose of 5-HT (mg/kg)	Time between 5-HT and ^{22}Na	Samples (no.)	Embryo and decidua capsularis (mean±S.E.)	Decidua basalis (mean±S.E.)	Endometrium and myometrium (mean±S.E.)
			Day 10		
Control	—	12	75±2	113±3	100±2
80	5	4	44±4†	76±5‡	98±3
80	30	6	12±1‡	46±5‡	60±12‡
10	5	6	66±2†	95±1†	90±3*
10	30	18	31±4‡	68±4‡	93±3*
10	90	6	54±7†	98±5*	102±1
			Day 11		
Control	—	12	68±2	98±2	92±2
80	5	4	30±4‡	61±6‡	81±4*
80	30	6	9±1‡	42±5‡	57±5‡
80	90	8	8±1‡	42±5‡	72±3‡
10	30	8	23±4‡	68±7‡	82±4*
10	90	8	34±3‡	82±4†	89±2

5-HT treated rats

The means, standard errors and significance levels for the ^{22}Na content of the tissues from the 5-HT treated rats are given in Tables 3 and 4.

TABLE 4

THE EFFECT OF 5-HT ON TISSUE ^{22}Na CONTENT (CPM/G × 100 ÷ CPM/ML. BLOOD) 5 MIN AFTER AN INTRAVENOUS INJECTION OF ^{22}NaCl. COMBINED RESULTS FOR DAY 10 AND 11

Significance determined by Student's *t* test each mean being compared with the control value. ($P<0{\cdot}05$*, $<0{\cdot}01$†, $<0{\cdot}001$‡).

Dose of 5-HT (mg/kg)	Time between 5-HT and ^{22}Na	Samples (no.)	Abdominal muscle (mean±S.E.)	Spleen (mean±S.E.)	Kidney (mean±S.E.)
Control	—	12	30±3	29±1	72±2
80	5	4	23±5	23±1*	61±2†
80	30	6	14±3†	18±3‡	64±2*
80	90	5	14±2†	14±2‡	63±3†
10	5	3	24±1	25±1	69±3
10	30	14	24±2	25±2*	61±1‡
10	90	8	26±1	32±1	69±3

It can be seen that in the presence of 5-HT the ^{22}Na content of the various tissues was significantly lower than the control values in nearly every case. For example, when a dose of 80 mg/kg of 5-HT was given on day 10, 30 min before the ^{22}Na injection, the ^{22}Na content of the embryo and decidua capsularis, and the decidua basalis samples were 12 ± 1 and 46 ± 5 respectively compared with control values of 75 ± 2 and 113 ± 3.

Since the control values varied in different tissues a direct comparison could not be made between the effect of 5-HT in one tissue and the effect in another, from the figures in these tables. In order to do this, and to illustrate the extent and characteristics of the inhibition, the difference in ^{22}Na content between control and 5-HT treated tissue—that is, the amount of ^{22}Na needed for complete equilibration—was expressed as a percentage of the control value. This value gave a measure of the inhibitory effect of 5-HT on ^{22}Na equilibration. Figure 3 illustrates the degree of the inhibition in the three portions of the uterus at (a) the two dose levels of 5-HT used and (b) the various time intervals between 5-HT and ^{22}Na administration.

5-HT was more effective in slowing ^{22}Na equilibration when the ^{22}Na was injected 30 or 90 min rather than 5 min after the 5-HT. The inhibition of the passage of ^{22}Na into the various tissues of the uterus increased progressively with the distance of the tissues from the myometrium (Fig. 3). Thus the effect of 5-HT was greatest in the embryo and decidua capsularis, where a dose of 80 mg/kg on day 10 or 11 respectively reduced the entry of ^{22}Na, given 30 min later, by 84 and 87%. Under the same conditions the effect of 5-HT was also quite marked in the decidua basalis (57 and 59% inhibition) and in the endometrium and myometrium (35 and 40% inhibition). With a dose of 10 mg/kg 5-HT the inhibitory effect was less in all tissues but again the inhibition was greatest in the embryo. Thus in the embryo and decidua capsularis there was 59 and 66% inhibition respectively on days 10 and 11 ; in the decidua basalis there was a 31 and 40% inhibition ; and in the endometrium and myometrium there was an 8 and 11% inhibition when ^{22}Na was given 30 min after the 5-HT.

There is some indication that the duration of the inhibitory effect was also proportional to the dose of 5-HT. For example, in the embryo and decidua capsularis on day 11, with a dose of 80 mg/kg, the inhibitory effect was just as great whether the ^{22}Na was given 30 min (82%) or 90 min (83%) after the 5-HT injection (Fig. 3). With a dose of

10 mg/kg on day 10, however, the degree of inhibition was significantly less when the ^{22}Na was injected 90 min after the 5-HT (28%) than when it was injected 30 min after (59%), although the value on day 11 at 90 min (51%) was not significantly less than that at 30 min (66%). A difference in duration of effect at the two dose levels was also seen in the decidua basalis, but the effects of even the high dose were less at 90 min in the endometrium and myometrium.

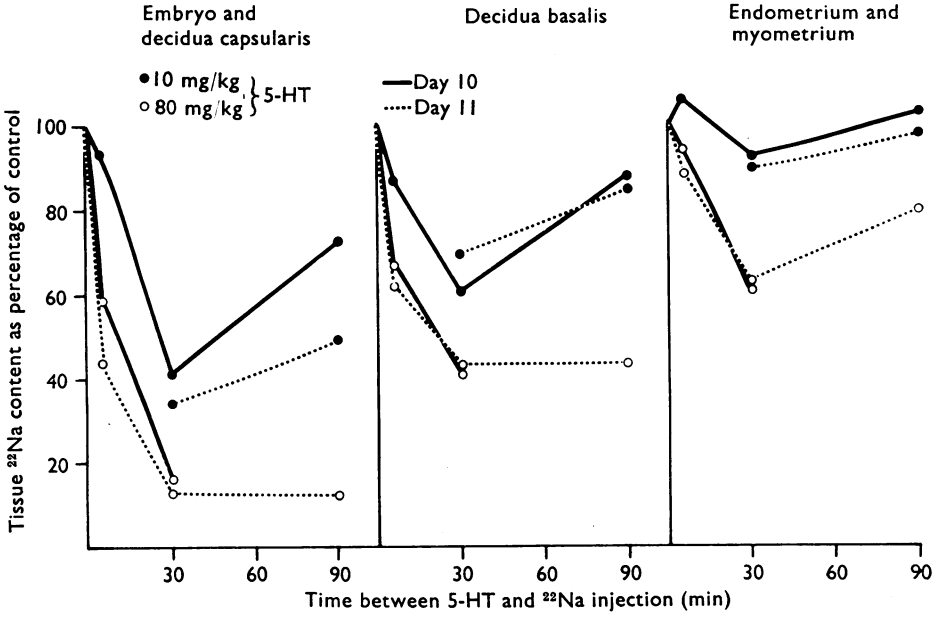

Fig. 3. Inhibition of equilibration of ^{22}Na with embryonic and uterine tissues produced by 80 and 10 mg/kg 5-HT. Tissues removed 5 min after the intravenous injection of ^{22}Na.

Some experiments, not described in Table 3, were performed to determine whether 5-HT still had any effect on sodium equilibration, when the animals were killed 30 min after ^{22}Na administration—that is, a period about six times longer than that required for full equilibration of sodium. The results showed that at this time such an effect could still be demonstrated in the embryo and decidua capsularis as well as in the decidua basalis. Thus when ^{22}Na was injected 30 min after the 5-HT—that is, when the effects of 5-HT was at its maximum—full equilibration did not occur in the embryo and decidua capsularis either on day 10 or 11, and the ^{22}Na contents were statistically lower than the control values (that is, 56 ± 2 compared with 71 ± 4 on day 10, $P<0.02$ and 32 ± 5 compared with 69 ± 2 on day 11, $P<0.001$). However, when the ^{22}Na was injected 5 min after the 5-HT injection (80 mg/kg), equilibration occurred within the 30 min period and the results for day 10 showed that the embryo and decidua capsularis contained significantly more ^{22}Na than the controls (85 ± 1 compared with 71 ± 4 respectively $P<0.001$).

As can be seen from Table 4 both doses of 5-HT (that is, 80 mg/kg and 10 mg/kg) significantly slowed the rate of equilibration of ^{22}Na with abdominal muscle, spleen and

kidney, particularly when given 30 or 90 min before the ^{22}Na injection. When 80 mg/kg 5-HT was given 5 or 30 min before the ^{22}Na injection, and the rats were killed 30 min later the spleens contained significantly more ^{22}Na than the control spleens—that is, 41 ± 2 compared with the control value of 31 ± 3 $P<0.02$, and 43 ± 3 compared with 31 ± 3 $P<0.05$. However, the muscle and kidney values were not significantly different from the control values.

DISCUSSION

These investigations have shown that during the period of embryogenesis a single injection of a large dose of 5-HT into the mother will kill the embryos on the same day. In smaller doses 5-HT can damage but not necessarily kill the embryos, and this leads to a very high proportion of the foetuses being damaged and showing abnormalities when examined at term. These results are in agreement with earlier findings in mice (Poulson *et al.*, 1963), though the rat seems to be somewhat more sensitive than the mouse to the action of 5-HT.

When 5–10 mg/kg 5-HT is given on day 10, many of the embryos die and many of the surviving foetuses have severe deformities. When these doses of 5-HT are given on day 11 the embryos usually survive and the foetuses at term have less severe abnormalities, although more of the foetuses are affected.

5-HT has been shown to cause gross abnormalities such as exencephalus, hydrocephalus and anophthalmia and omphalocele in rats (Reddy *et al.*, 1963). In the present investigation no cases of omphalocele were seen but there were additional gross deformities such as shortened snouts and missing digits. However in the investigation of Reddy *et al.* 5-HT was given daily in a divided dose throughout the whole of the pregnancy. From the present study it appears that a single injection of 10 mg/kg 5-HT on the afternoon of day 10 produced almost the same damage as daily injections of 5 mg/kg 5-HT throughout the whole of pregnancy as reported by Reddy *et al.* (1963).

There appears to be no previous report on the production of certain abnormalities, viz., fusion of the vertebrae and ribs, resulting from the administration of 5-HT (5–10 mg/kg). In the present investigation they were seen in a large proportion of the foetuses. However, they have been shown to occur in mice where the mother was subjected to anoxia (Ingalls *et al.*, 1952) and in mice treated with 5-HT (Robson & Sullivan, unpublished).

The second part of the investigation involved a study of the mechanism by which 5-HT produced these effects. It was found that, in doses which caused embryonic death (80 mg/kg) or which caused embryonic damage (10 mg/kg), 5-HT slowed the passage of ^{22}Na into all the tissues investigated. The effect was greatest in the embryonic tissue. Here, as in other tissues, the effect was maximal 30 min after the subcutaneous injection of 5-HT. It is of interest that Senior (1966) who investigated the 5-HT content of the blood at various times following a subcutaneous injection of 5-HT, found that the maximum concentration occurred 30–60 min later and thereafter there was a slow decrease.

5-HT produced about the same degree of inhibition of the passage of ^{22}Na into the various tissues whether the experiments were performed on day 10 or on day 11. Thus it seems unlikely that the different types of abnormalities produced by 10 mg/kg on day

10 compared with day 11 were related to differences in ^{22}Na equilibration on these two days. It seems much more likely that the risk to the various organs was related to their stage of development thus making them susceptible to nutritional starvation on these days (Beck & Lloyd, 1965). It is interesting to note that other maternal tissues did not appear to be permanently damaged by these doses of 5-HT.

There was a greater inhibition of the passage of ^{22}Na into the various tissues with 80 mg/kg than with 10 mg/kg. With the higher dose the effect on the embryo and decidua capsularis was still maximal even after 90 min, and therefore it lasted for a considerably longer period than this, whereas with the other dose the effect was less at 90 min and, extrapolating from Fig. 3, appeared to last for about $2\frac{1}{2}$ hr.

If the equilibration of ^{22}Na in tissues can be considered as a reliable index of what would happen with other nutrients then it would appear that the embryo is virtually "cut off" from its source of nutrients by embryotoxic doses of 5-HT. If this argument is valid then it can be concluded that the teratogenic dose of 5-HT (10 mg/kg), on day 10 or 11, interferes with the supply of nutrients to the embryo for approximately $2\frac{1}{2}$ hr and at maximum produces a 60% inhibition. However, the lethal dose of 5-HT (80 mg/kg) causes a greater maximal inhibition—85%—and the effect apears to last for longer than $2\frac{1}{2}$ hr (e.g., still maximal after $1\frac{1}{2}$ hr) (Fig. 3).

In this connection it is interesting to note the findings of Brent & Franklin (1960) who showed, in the rat, that when the uterus is clamped and completely isolated from the maternal circulation on what corresponds to our day 10 of pregnancy, none of the surviving foetuses examined at term were abnormal after $\frac{1}{2}$–1 hr period of clamping whereas 8/48 of the surviving foetuses were malformed after $1\frac{1}{2}$–3 hr of clamping.

We thus conclude that the mechanism by which 5-HT kills or damages the embryo during the period of embryogenesis involves a decrease in the supply of nutrients to the embryonic tissue, but the exact site of action is not clear.

At a later stage of pregnancy in rats, Craig (1966) concluded that 5-HT terminated pregnancy by constricting the major uterine blood vessels. In the present study, an action solely on the mesometrial blood vessels could not account for the inhibition of ^{22}Na equilibration which occurs in the uterus, for on some occasions there was very little or no inhibition in the endometrium and myometrium even while there was a marked inhibition in the decidua basalis and embryo and decidua capsularis. The inhibitory effect in the uterus also became progressively greater the more distant the tissue was from the myometrium. Therefore this suggests that the small blood vessels passing through the endometrium into the decidua basalis and decidua capsularis, and supplying the yolk-sac placenta, are also sensitive to 5-HT, and that additional constriction here effectively isolates the embryonic tissue from its source of nutrients. This still requires further investigation in view of the findings, at later stages of pregnancy, that 5-HT may have an effect on tissue permeability as well as blood flow (Honey, Robson & Sullivan, 1967).

SUMMARY

1. When rats were given a single subcutaneous injection of 20 mg/kg or more of 5-HT creatinine sulphate on day 10 or 11 of pregnancy no embryos survived to term.

2. When given a lower dose—that is, 10 mg/kg—some embryos survived, and with 2 mg/kg all the embryos survived. In this dose range a large proportion of the surviving foetuses were abnormal when examined at term. The type of abnormality produced was largely dependent on the day of administration—that is, day 10 or 11.

3. It was shown, that, under normal conditions, ^{22}Na equilibrated with the body tissues within 5 min of its intravenous injection on day 10 and 11 of pregnancy. When 5-HT was injected subcutaneouly at certain times before the ^{22}Na injection the rate of equilibration of ^{22}Na was slowed in all the tissues investigated. The slowing was particularly marked in the uterine tissues, becoming progressively greater the more distant the tissue was from the myometrium—that is, the effect was greatest in the embryo and decidua capsularis. Here, with a dose of 10 mg/kg 5-HT given 30 min before the ^{22}Na, equilibration took some 30 times longer. The effect was even more marked with a larger dose of 5-HT.

4. If it is true that other nutrients are similarly prevented from equilibrating with the embryonic tissue by 5-HT, then it appears that this is the mechanism by which 5-HT produces its lethal or teratogenic effects. As there is no chorioallantoic placenta at this stage and since the effect becomes progressively greater the more distant the embryonic tissue is from the myometrium it would appear that 5-HT has a direct action on the blood vessels within the implantation site as well as on the mesometrial vessels.

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